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THE SHIRAIACHROMES: NOVEL FUNGAL PERYLENEQUINONE PIGMENTS FROM *SHIRAIA BAMBUSICOLA*

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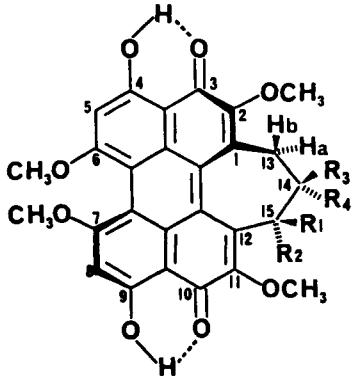
ABSTRACT.—Three novel perylenequinone pigments shiraiachromes A, B, and C [**1–3**] have been isolated from the Chinese bamboo fungus, *Shiraia bambusicola*. Their structures have been determined on the basis of spectroscopic data and chemical correlations.

The mycelium of a number of phytopathogenic fungi shows deep red pigmentation due to the presence of secondary metabolites containing a perylenequinone nucleus (1–3). These fungal metabolites exert photodynamic activity towards bacteria and fungi, which is evidenced by their inhibition of the growth of other microorganisms upon irradiation. This interesting biological activity arising from the chromophore is related to cellular lipid oxidation and has been used as a phototherapeutic agent for some dermatomycoses (4). In the course of searching for new natural perylenequinone pigments, we have isolated three new related metabolites, shiraiachromes A–C [**1–3**], from the fungus *Shiraia bambusicola* P. Henn (Hypocreaceae) which grows on bamboo. Their structures are closely related to hypocrellin [**4**] which was isolated from another bamboo fungus *Hypocrella bambusae*. We report their structures here.

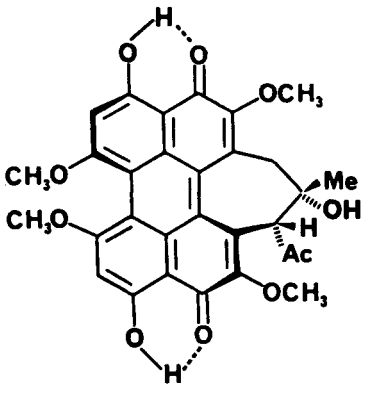
RESULTS AND DISCUSSION

The mycelium of the fungus *S. bambusicola* collected in Szechuan, China, was extracted with Me_2CO. The Me_2CO extract was fractionated on a Sephadex LH-20 column, and the pigment fraction was further separated and purified by successive reversed-phase hplc using ODS column to give shiraiachromes A, B, and C [**1–3**].

Shiraiachrome A [**1**], deep red crystals, displayed a molecular ion at *m/z* 546.1535 in agreement with a molecular formula $C_{30}H_{26}O_{10}$. A perylenequinone nucleus was



1 R_1=H, R_2=Ac
R_3=Me, R_4=OH
4 R_1=Ac, R_2=H, R_3=OH, R_4=Me



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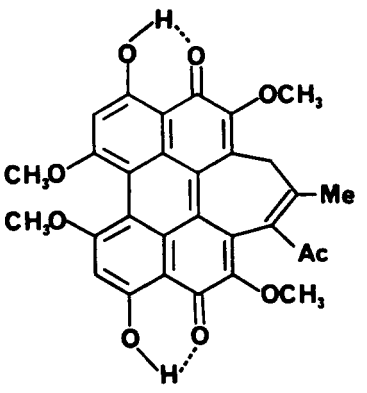

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evident from uv (λ max at 578, 537, 463, and 340 nm) and ir (ν max at 1610, 840, and 3420 cm^{-1}) spectra as well as the following ^1H nmr signals: δ 15.98 and 16.10 (for chelating protons of perylenequinone), 6.56 and 6.57 (for H-5 and H-8) and 4.08, 4.08, 4.19, and 4.28 (for four aromatic methoxy groups). The remaining ^1H-nmr signals included two singlet methyl signals at δ 1.79 and 1.84, two geminal proton signals at δ 2.35 and 3.68 (d, $J = 14$ Hz each) and a singlet proton signal at δ3.74. In addition, its eims showed main fragments at *m/z* 528 $[M-H_2O]^+$, 513 $[M-H_2O-CH_3]^+$, 497 $[M-H_2O-OMe]^+$, and 485 $[M-H_2O-Ac]^+$. These data are consistent with a seven-membered side ring structure similar to that of hypocrellin [**4**] (3). The gross structure of **1** was proved to be the same as that of **4** by comparing their spectroscopic data and their chemical conversion into shiraiachrome C [**3**]. The cd spectrum (MeOH) of **1** possessed positive Cotton effects at 588, 540, and 350 nm and negative Cotton effects at 450, 283, and 255 nm, which were parallel to those of **4** except for a small but very clear positive Cotton effect at 295 nm (Figure 1). These data revealed that **1** possessed a twisted perylenequinone moiety and must have the same axial chirality as **4**, i.e., *R*, that of **4** having been determined by correlation to that of cercosporin (5). On the other hand, the positive Cotton effect at 295 nm reflected the difference of orientation of the acetyl group (15-Ac) between **1** and **4**. In **4** the acetyl group is situated β and adopts equatorial orientation, but in **1** the acetyl group must be α and oriented axially, which was further confirmed by the chemical shift value of H-15: it is 3.74 ppm, 0.27 ppm lower than that of **4**. This clearly suggested that H-15 of **1** is in the β position and oriented equatorially. Finally, the stereochemistry of C-14 can be deduced by comparing relevant chemical shifts of H-13a and H-13b between **1** and **4** (Table 1). The low field shift (0.15 ppm) of H-13a and high field shift (0.29 ppm) of H-13b in **1** from those of **4** indicate the orientation of the hydroxy group (14-OH) to be as shown in **1**.

Shiraiachrome B [**2**], deep red crystals, is less polar than **1**. It is identical to **4** in all respects including uv, ir, eims, ^1H nmr, tlc behavior, and feasible conversion to **3** on treatment with alkali. However, its cd spectrum was opposite to that of **4** (see Figure 1); hence it can be assigned as the antipode of **4**.

Shiraiachrome C [**3**] showed uv (λ max at 588, 550, 461, and 334 nm) indicating the presence of the same perylenequinone nucleus. Its eims displayed a molecular ion at *m/z* 528, 18 amu lower than that of **1** and **2**. In agreement, the ^1H-nmr spectrum suggested the absence of H-15, and the ^{13}C-nmr signal at δ 200.1 indicated C-17 to be an unsaturated ketone, which was confirmed by the ir band 1689. These spectroscopic data revealed that **3** is the dehydration product of **1** and **2**, which was confirmed by the chemical conversion from **1**, **2**, and **4** to **3**.

TABLE 1. The ^{1}H-nmr Data (200 MHz) of Compounds **1**, **2**, **3**, and **4** ($CDCl_3$, TMS).[a]

Proton	Compound			
	1	**2**	**3**	**4**
H-5	6.56, s	6.55, s	6.43, s	6.55, s
H-8	6.57, s	6.57, s	6.44, s	6.57, s
H-13a	3.68, d, 14	3.52, d, 12	4.05, d, 11.5	3.52, d, 12
H-13b	2.35, d, 14	2.64, d, 12	3.22, d, 11.5	2.64, d, 12
H-15	3.74, s	3.47, s	—	3.47, s
4-OH	15.98, s	15.95, s	16.03, s	15.95, s
9-OH	16.10, s	15.99, s	16.05, s	15.98, s
2-OMe	4.19, s	4.08, s	4.09, s	4.08, s
6-OMe	4.08, s	4.07, s	4.05, s	4.07, s
7-OMe	4.08, s	4.07, s	4.05, s	4.07, s
11-OMe	4.28, s	4.12, s	4.15, s	4.12, s
14-Me	1.79, s	1.71, s	1.84, s	1.71, s
15-Ac	1.84, s	1.90, s	2.38, s	1.90, s

[a]δ in ppm, multiplicity, J in Hz.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were determined on a Reichert hot stage apparatus and are uncorrected. Uv and ir spectra were recorded on Shimadzu UV365 and Perkin-Elmer 983 instruments, respectively. ^{1}H-nmr spectra were recorded on a Varian XL-200 spectrometer with TMS as internal standard. ^{13}C-nmr spectra were taken on a JEOL FX90Q instrument running at 22.5 MHz. Eims spectra were obtained with a Finnigan-4021 spectrometer operating at 70 eV, and hrms was measured with a Finnigan MAT instrument. Cd spectra were recorded on a JASCO J-500 instrument.

FUNGUS MATERIAL.—The mycelium of the fungus *S. bambusicola* was collected in Szechuan, China, in 1984. The identity of the fungus material was authenticated at the South China Institute of Botany, Guangzhou, where a voucher specimen was deposited.

ISOLATION PROCEDURE.—The mycelium (295 g) was extracted with Me_2CO (2 liters × 5). The solvent was evaporated under reduced pressure to give a crude extract (40 g). The extract was crystallized from Me_2CO to give deep red crystals. A part of the crystals (100 mg) was fractionated on a Sephadex LH-20 column (20 × 800 mm) using $CHCl_3$-MeOH (1:1) as eluent, and the pigment fraction was collected (60 mg). It was chromatographed on a C_{18} column (YMC Pack AM 312 Yamamura Chemical, 5.7 × 150 mm) with an MeOH-H_2O (85:15) eluent to yield shiraiachrome A [**1**] (Rt 6.1 min, 27.2 mg), shiraiachrome B [**2**] (Rt 6.8 min, 24.4 mg), and shiraiachrome C [**3**] (Rt 8 min, 5.1 mg).

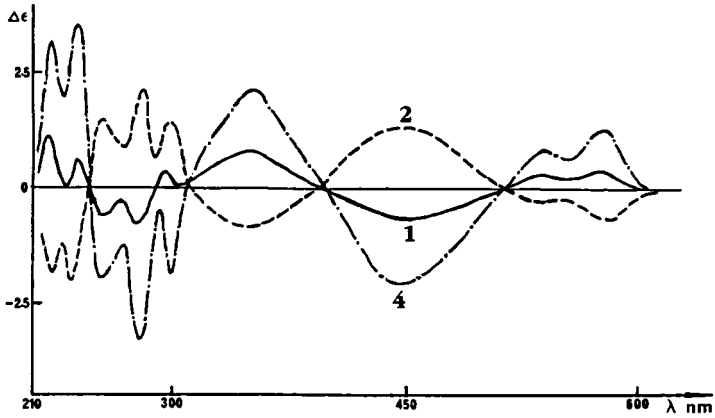

FIGURE 1. The cd spectra of compounds **1** (——), **2** (-----), and **4** (–·–·–).

SHIRAIACHROME A [**1**].—Mp 247–250°; uv λ max (MeOH, log ε) 580 (4.09), 540 (4.07), 465 (4.41), 338 (3.51), 285 (4.48), 265 (4.56), 215 (4.74); ir (KBr) 3415, 2939, 1700, 1608, 1529, 1451, 1285, 1215, 1160, 997, 968, 835, 668, 589, 532 cm^{-1}; ^{13}C nmr ($CDCl_3$, TMS) δ 24.8, 28.5, 42.5, 56.4, 56.4, 61.0, 61.8, 64.1, 78.6, 101.6, 102.0, 106.6, 106.9, 124.3, 124.3, 127.7, 127.7, 131.1, 134.9, 149.0, 151.6, 167.1, 167.5, 171.5, 171.5, 179.1, 179.4, 179.5, 179.9, 206.5; ms *m/z* (rel. int.) 546 (1.9), 528 (46), 513 (15), 497 (16), 485 (23), 455 (13); hrms *m/z* 546.1535 (calcd for $C_{30}H_{26}O_{10}$, 546.1526).

SHIRAIACHROME B [**2**].—Mp 247–248°; uv λ max (MeOH) 580 (4.12), 540 (4.09), 465 (4.42), 340 (3.75), 283 (4.50), 267 (4.55), 215 (4.74); ir (KBr) 3423, 2939, 1702, 1609, 1528, 1451, 1823, 1217, 1161, 1091, 1050, 997, 971, 950, 835 cm^{-1}; eims *m/z* 546 (11), 528 (10), 513 (4), 497 (4), 485 (15), 471 (3), 457 (7), 445 (8).

SHIRAIACHROME C [**3**].—Mp 278–280°; uv λ max (MeOH) 587 (4.09), 550 (4.24), 461 (4.57), 334 (4.09); ir (KBr) 3397, 1689, 1609, 1523 cm^{-1}; ^{13}C nmr ($CDCl_3$, TMS) δ 20.55, 29.33, 34.64, 56.41 × 2, 60.97, 61.18, 102.90 × 2, 107.23, 108.42, 121.75, 123.05, 123.81 × 2, 133.99, 124.32 × 2, 144.61, 146.56, 149.38, 163.55, 164.87, 167.90, 168.12, 185.46 × 4, 200.08; eims *m/z* $[M]^+$ 528 (53), 513 (17), 497 (15), 485 (19), 455 (7); hrms *m/z* 528.1409 (calcd for $C_{30}H_{24}O_9$, 528.1420).

DEHYDRATION OF SHIRAIACHROME A [**1**], SHIRAIACHROME B [**2**], AND HYPOCRELLIN [**4**].—Shiraiachrome A [**1**] (5.2 mg) was treated with KOH (3%, 5 ml) at room temperature for 5 h. The reaction mixture was neutralized with HCl (10%) and extracted with $CHCl_3$ (10 ml × 3). The $CHCl_3$ was removed under vacuum, and the residue was dissolved in CH_2Cl_2 and passed down a short Si gel column to yield a deep-red solid (4.9 mg) which was identical to shiraiachrome C [**3**] in ^1H nmr, eims, and tlc behavior.

Shiraiachrome B [**2**] (4.6 mg) was treated with KOH (1.5%, 3 ml) and worked up as above to yield shiraiachrome C [**3**].

Hypocrellin [**4**] (5.0 mg) was treated with KOH (1.5%, 3 ml) and worked up as above to afford shiraiachrome C [**3**].

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